PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 31/445, 31/70, 38/13, 9/06, 47/12, 31/435

A1

(11) International Publication Number: WO 99/24036

(43) International Publication Date: 20 May 1999 (20.05.99)

(21) International Application Number: PCT/GB98/03317

(22) International Filing Date: 5 November 1998 (05.11.98)

(30) Priority Data:
9723669.9 7 November 1997 (07.11.97) GB

(71) Applicant *(for all designated States except US)*: ABERDEEN UNIVERSITY [GB/GB]; Auris Business Centre, 23 St. Machar Drive, Aberdeen AB2 1RY (GB).

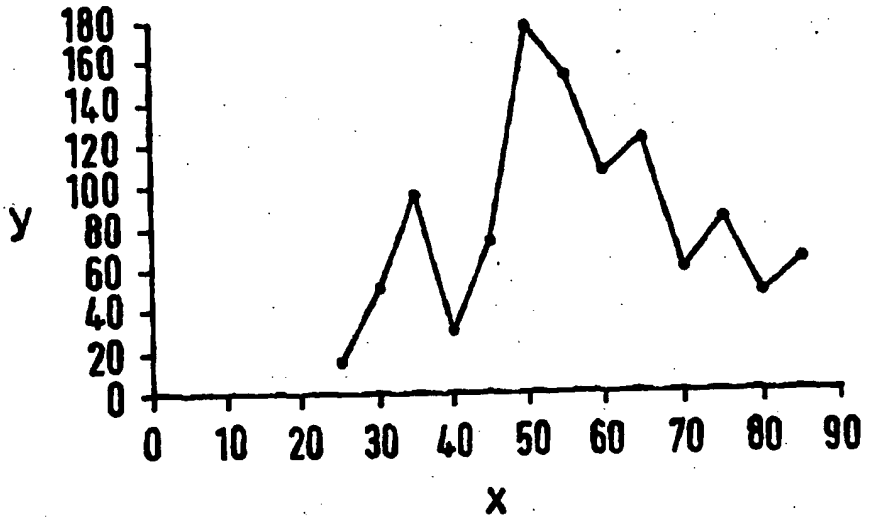
(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: ORMEROD, Anthony, David [GB/GB]; 12 Kemnay Place, Aberdeen AB15 8SG (GB). WINFIELD, Arthur [GB/GB]; 42 Westholme Avenue, Aberdeen AB15 6AB (GB).

(74) Agents: STEBBING, Peter, John, Hunter et al.; Ablett & Stebbing, Caparo House, 101–103 Baker Street, London W1M 1FD (GB).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: SKIN PENETRATION ENHANCING COMPONENTS



(57) Abstract

The present invention relates to a topical formulation for the treatment of a dermatological condition which comprises a macrocyclic lactone antibiotic, immunosuppressive macrolide or a biologically active analogue, derivative or pro–drug thereof; characterized in that it further comprises a permeation modulator and the permeation modulator and the macrocyclic lactone or macrolide or the biologically active analogue, derivative or pro–drug thereof are present in relative amounts such that when a therapeutic amount is applied to the skin a minimal systemic effect is produced. The immunosuppressive macrolide may be sirolimus.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia and Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CU	Cuba
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GH	Ghana
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IL	Israel
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
MK	The former Yugoslav Republic of Macedonia
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

SKIN PENETRATION ENHANCING COMPONENTS

This present invention relates to an effective treatment for psoriasis and other dermatological conditions using a topically applied immunosuppressive agent. The preferred formulation does not allow the agent to appear in the blood or other circulatory system at any significant level.

Dermatological conditions can be uncomfortable and embarrassing for the patient, so an effective safe treatment is required. Some dermatological conditions are caused by an overactive immune system, examples are psoriasis, alopecia, lichen planus, lupus erythematosus, pyoderma gangrenosum, vitiligo and graft versus host disease. Others can be due to bacterial or pustular skin infections.

Dermatological conditions caused by an overactive immune system can be treated by immunosuppressive macrolides, for example sirolimus (rapamycin), FK-506 (tacrolimus) or SDZ ASM 981. Those that are caused by bacteria or are deeper skin infections, such as acne vulgaris and hidranitis suppcurativa, can be treated by macrolide antibiotics, for example erythromycin, azithromycin and clarithromycin. The above agents may be applied by means of topical creams and lotions or taken orally.

Psoriasis affects 2.4% of the population and the current understanding of the pathogenesis of the disease is that it is driven initially by immunocytes. These and keratinocytes are mutually stimulated and activated through the production of cytokines, TGFa, IL-6 and IL-8 from lymphocytes. This leads to a hyperproliferative epidermis with rapid 36 hour cycling of the transient amplifying compartment of

keratinocytes.

FK506 is a macrolide antibiotic which shows part homology with sirolimus. Research in models has shown that it has some efficacy in the topical therapy of contact dermatitis, atopic eczema and to a lesser degree psoriasis. Cyclosporin is also known to be effective in treating a wide range of skin diseases. However the usefulness of these drugs is limited by their potential side effects resulting from systemic administration.

Other forms of treatment of dermatological conditions may include using topical steroids but these have undesirable effects such as irreversible atrophy and purpura.

In the treatment of the human or animal body, one of the considerations is that any medicament shall as far as possible affect only the afflicted part. It is well known that amounts of circulating drug should be kept as low as possible to avoid unwanted mutations. A problem with the topical application of medicaments to the skin for example, is that the medicament tends to penetrate the skin and establish itself in the circulating blood system. This is not what is intended in the treatment of dermatological conditions.

The macrocyclic lactone antibiotic rapamycin for example as disclosed in EP-A-0533433 has already been used topically to treat such skin disorders as psoriasis and dermatitis. However no attempt has been made to reduce the amount of rapamycin translocated across the skin into the systemic system. Nor is there any discussion of the reduction of the levels of circulating rapamycin or other macrolide drug at the same time as providing therapeutically effective treatment for

a variety of skin disorders.

We have now found that this may be achieved by the addition to such drugs of a permeation modulator. Permeation enhancers are well known as a class of drug translocation facilitors, but the purpose of these is to increase the drug flux across the skin. A permeation modulator however has the facility to allow the drug to penetrate the skin, and particularly the stratum corneum, without significantly passing through the epidermis into systemic systems (eg the blood or lymph systems).

It is also known that immunosuppressive agents taken orally and steroids applied topically can be used to treat dermatological conditions, such as psoriasis or eczema. However, they are often non-specific in their action which leads to undesirable side effects. Thus it would be desirable to develop a topical delivery formulation for an immunosuppressive agent which preferentially treats the diseased sites only and avoids significant systemic exposure; so reducing harmful side effects.

Sirolimus is a macrocyclic lactone antibiotic produced by the organism *Streptomyces hygroscopicus;* it is known to have potent immunosuppressive activities. Sirolimus acts through specific binding of a family of cytosolic immunophilins called the FK binding proteins (FKBP). The sirolimus FKBP complex acts at least three sites. Firstly, by blocking the phosphorylation activation of p70 s6 kinase, an enzyme acting on the 40S ribosomal subunit s6 protein, thereby reducing the efficiency of translation. Secondly by preventing activation of specific elongation factors required for protein synthesis. Thirdly, it inhibits enzyme activity of the cyclin dependent

kinase cdK-cyclin E complex which forms one of the tight controls of the G1/S transition in cell division by inhibiting the normal decline of the p27 cdk inhibitor which would follow IL-2 stimulation. Sirolimus has an advantage over other immunosuppressive agents in the treatment of psoriasis as it has an inhibitory effect on keratinocyte proliferation. *In vitro* experiments have shown that this inhibitory effect takes place at concentrations ranging from 3-10μg/ml. A broader range may be employed for example 1 to 20μg/ml, but the more efficacious range is 5-8μg/ml.

According to the first aspect of the invention, there is provided a topical formulation for the treatment of a dermatological condition which comprises a macrocyclic lactone antibiotic or immunosuppressive macrolide or a pharmacologically active analogue, derivative or pro-drug thereof; characterised in that it further comprises a permeation modulator and the permeation modulator and the macrocyclic lactone antibiotic, immunosuppressive macrolide or pharmacologically active analogue, derivative or pro-drug are present in relative amounts such that when a therapeutic amount is applied to the skin, a minimal systemic effect is produced.

By the term "minimal systemic effect", is meant that the amount of active principal detectable in the blood stream is preferably less than 0.3 ng/nl over 4 to 24 hours after administration, more preferably below 0.1 ng/ml over the same period.

Preferably the macrocyclic lactone antibiotic is selected from erythromycin, azithromycin or clarithromycin. These macrocyclic lactone antibiotics are effective for treating

pustular and bacterial skin infections such as acne vulgaris.

Conveniently the immunosuppressive macrolide is selected from sirolimus, FK-506 or SDZ ASM 981. Sirolimus is a favoured alternative because it is also an effective antibiotic which is useful in the microbiological preservation of the formulation. The microbiological properties of sirolimus are also helpful in the treatment of scalp and flexural psoriasis, seborrhoeic dermatitis and in secondarily atopic eczema.

In preferred embodiments the permeation modulator may be an alkanoic or alkenic acid, preferably having 6 to 20 carbon atoms such as capric acid, octanoic acid, oleic acid or acids or such acids of intermediate chain length. The permeation modulator aids the penetration of the immunosuppressive macrolide or macrocyclic antibiotic through the stratum corneum, the principle barrier to the penetration of drugs. The stratum corneum is an aggregate of the stacked, flattened skeletons of keratin filled cells interspersed with lipid monolayer structures and water. The addition of the permeation modulator to the formulation results in the partial disruption of the barrier components, particularly the lipid structures. A gradient of the drug can then be produced across the stratum corneum particularly, which facilitates the diffusion of the immunosuppressive macrolide or macrocyclic lactone antibiotic across the stratum corneum into the living epidermis. The relative concentrations of the macrolide or antibiotic and the permeation modulator are chosen so that only partial penetration of the skin occurs; the macrocyclic lactone antibiotics or immunosuppressive macrolides reach the areas which require treatment but significant absorption of the said drugs into the systemic circulation is avoided thus reducing the likelihood of any systemic side effects.

Conveniently the permeation modulator is used in conjunction with a solvent system which includes an aromatic alcohol such as phenyl-alkanol or a biologically acceptable benzene derivative, with or without an admixture of monoglycerides and/or a fatty acid ester (e.g. isopropyl myristate). Other solvents used, include benzaldehyde, benzyl benzoate and acetone. The combination of solvent and permeation modulator further optimises the passage of the immunosuppressive macrolide or the macrocyclic lactone antibiotic across the stratum corneum.

Preferably, the concentration of the macrocyclic lactone antibiotic or immunosuppressive macrolide is up to 10% by weight of the formulation. More preferably the concentration of the macrocyclic lactone antibiotic or immunosuppressive macrolide is either 0.5% to 5.9% or 6% to 12% by weight. Even more preferably the concentration of the macrocyclic antibiotic or immunosuppressive macrolide is either 1 to 5% or 6 to 8% by weight. A concentration of 0.05% to 2% is most preferable in the treatment of eczema. The term "% by weight" used herein refers to the "% by weight of the final formulation".

Preferably the above ranges of macrocyclic lactone antibiotic or immunosuppressive macrolide or analogue derivative or pro-drug thereof are used in an agent comprising a permeation modulator; wherein the concentration of the permeation modulator is 0.1% to 60% by weight. More preferably the concentration of the permeation modulator is either 0.1% to 39.9% or 40% to 80% by weight. Even more preferably the concentration of the permeation modulator is either 0.1% to 19.9%, 20% to 39.9% or 40% to 60%.

Preferably the above ranges of macrocyclic lactone antibiotic or immunosuppressive and permeation modulator are used in a formulation in conjunction with a solvent system; wherein the concentration of the solvent system is 5% to 90% by weight. More preferably the concentration of the solvent system is either 0.1% to 49.9% or 50% to 90% by weight. Even more preferably the concentration of the solvent system is either 0.1% to 19.9%, 20% to 39.9%, 40% to 69.9% or 70% to 90% by weight.

Preferably a thickening agent is present in the formulation. If the formulation is to be used topically, it should be of an appropriate consistency. Therefore, thickening agents such as cetostearyl alcohol or commercially available medical grade white soft paraffin may be added. These can reduce the penetration of the immunosuppressive agent but they are required for effective application. The formulations of the invention are particularly suitable for treatment of conditions of the scalp.

In addition to the liquid and solid vehicles set forth above, the formulations of the invention may additionally include one of the following:- flavouring agents, lubricants, solubilizers, suspending agents, filler and glidants.

The formulation can also be dissolved or suspended in any pharmaceutically acceptable liquid carrier or vehicle such as water or a pharmaceutically acceptable oil or fat. Such a liquid carrier or vehicle can contain other pharmaceutically acceptable additives such as solubilizers, emulsifier, buffers, preservatives, suspending agents, thickening agents, colouring agents, viscosity regulators, stabilizers or osmo-regulators.

The invention will now be described, by way of illustration only, with reference to the following examples, tables and figures accompanying the specification

Figure 1 is a graphical representation of the effect on the flux (µg/hr/cm^2) of sirolimus (y) through the stratum corneum by varying the capric acid and benzyl alcohol ratio, where x is the percentage of capric acid in the benzyl alcohol.

Figure 2 is a graphical representation of the effect on the flux (µg/hr/cm^2) of sirolimus (y) through the stratum corneum by varying the octanoic acid and benzyl alcohol ratio, where x is the percentage of octanoic acid in the benzyl alcohol.

Figure 3 is a graphical representation of the effect on the flux (µg/hr/cm^2) of sirolimus (y) through the stratum corneum by varying the oleic acid and benzyl alcohol ratio, where x is the percentage of oleic acid in the benzyl alcohol.

Figure 4 is a graphical representation of the effect on the flux (µg/hr/cm^2) of sirolimus (y) through the stratum corneum by varying the sirolimus concentration (mg/ml) (x) while keeping the capric acid to benzyl acid ratio constant.

Figure 5 is a graphical representation of the results of the clinical score (y) determined after application of the sirolimus formulation ( ) and the control (⁝⁝⁝) in Example 3.

Figure 6 is a graphical representation of the difference in the clinical score after application with sirolimus formulation in Example 3, where y is the number of subjects

in each group. A positive score (x) shows improvement with use of the active formulation.

Figures 1 to 4 were obtained by *in vitro* experimentation. The results were used to optimize the sirolimus concentration and the ratio of permeation enhancer and solvent used in *in vivo* experiments.

Example 1

A formulation was formed of 8% sirolimus and 92% of a vehicle of capric acid (50%) with benzyl alcohol (50%). This was tested in single application experiments on four individuals with normal skin. Venous blood samples were taken at 4, 7 and 24 hours after application and no significant levels of sirolimus were detected using MSGCMS, which is able to detect sirolimus levels down to 0.1ng/ml.

In parallel, skin biopsies were taken from the individuals after 7 hours, the biopsy samples were glued to a glass slide and serially sectioned horizontally into 4 layers each 0.7mm thick and extracted with acetonitrile. The results are given in Table 1.

Table 1 shows the tissue concentrations of sirolimus 7 hours after application of capric acid: benzyl alcohol (50:50) containing sirolimus at 8%. The horizontal skin sections were each 0.7mm. Accordingly, for example, the section of skin designated 2 was the horizontal layer of skin 0.7-1.4mm from the surface of the skin.

Section of skin 1=surface	Sirolimus concentration µg/mg			
	A	B	C	D
1	0.059	0.288	0.301	0.216
2	Not done	0.108	0.144	0.126
3	0.255	0.173	0.339	0.256
4	0.239	0.214	0.370	0.241

Example 2

A formulation of sirolimus (2.2%) in a vehicle comprising isopropyl myristate 40%, benzyl alcohol 10% and capric acid 50% was tested in single application experiments on three individuals with normal skin. Venous blood samples were taken at 4, 7 and 24 hours after application and no significant levels of sirolimus were detected using MSGCMS.

After 7 hours biopsy samples were taken from two of the individuals. These were bisected in parallel with the surface to give an upper and lower half, roughly corresponding to the epidermis and dermis. The skin was homogenised with acetonitrile and sirolimus concentration was determined by HPLC. The results are given in Table 2

Table 2 shows the tissue concentrations of sirolimus 7 hours after application of capric acid: isopropyl myristate: benzyl alcohol (50:40:10) containing sirolimus at 2.2%.

Level of skin segment	Sirolimus Concentration µg/mg	
	Subject A	Subject B
Upper (1)	0	1.5
Lower (2)	0.333	0.5

Example 3

A double blind, left-right comparison of the effect of applying topical sirolimus in formulations as described in Examples 1 and 2, to 24 patients with chronic (over three months) plaque psoriasis was conducted. (22 out of the 24 patients were eventually analysed.) A single target plaque was treated for the first 6 weeks with the lower potency formulation of Example 2. After this the active treatment was increased to the higher potency formulation of Example 1 for 6 weeks unless a clear improvement on one side had already occurred.

The study included adults with stable, clearly demarcated, chronic plaque psoriasis, and two, well matched, contralateral, comparable plaques about 50cm^2 in area on opposite sides of the body. Subjects were all aged over 18 years, were able to apply creams and had no other significant medical problems. Transaminases were not more than twice the upper limit of normal and subjects were selected to avoid those likely to have a holiday in sunlight during the 6-12 weeks of the trial.

Before the trial started, there was a two week washout period in which only bland emollients were applied to the target lesions.

Treatment was randomised and double blind. Hands were thoroughly washed between the twice daily application of the test formulations. The active formulation was applied consistently to one plaque while a control comprising only the vehicle base was applied consistently to the plaque on the opposite side. Where possible the arms or elbows were selected as target areas as cross contamination is less likely at these

sites.

Assessments were done at weeks 0, 2, 4 and 6 on the low potency treatment and at 8,10 and 12 on the higher dose formulation, provided there were no signs or laboratory evidence of toxicity. Clinical scoring was done at each attendance and areas traced at the start and finish of treatment. Biopsies from active and control lesions were performed at the end of treatment or at withdrawal. Biopsies were not done if an adverse event such as a reaction to the application occurred as this would influence the measures being assessed.

The lesions were also assessed at fortnightly intervals with subjective scoring on a scale of 0-8 for erythema, thickening, and scaling. Objective measures of improvement were performed on both lesions at the end of each treatment period (low and high formulations). These included pulsed A scan ultrasound measurement of lesion thickness and erythema measured with a reflectance erythema metre, both were averaged over 5 areas in each psoriatic lesion and were validated using a previous study which was performed using betamethasome as a reference.

At each visit we measured the full blood count, biochemistry, including urea, electrolytes, liver enzymes, bilirubin, calcium, magnesium, uric acid, glucose, amylase, muscle enzymes, lipids and cholesterol. Sirolimus levels were performed every 2 weeks during therapy. Samples for sirolimus levels were stored at minus 80° C and shipped to a central reference laboratory for analysis by LC/MS/MS by Wyeth Ayerst Research.

In biopsies, epidermal thickness was measured and

immunoperoxidase immunohistochemistry done using the following antibodies to count cells in a blinded fashion:

Thus, antibody Ki-67 was used to give a measure of hyperproliferation in the epidermis and CD4 helper lymphocytes were used to give a measure of auto-immune activity which drives psoriasis.

Cell counting in tissues was automated, using computer assisted image analysis (Seescan). Data was analysed by Student's T test for paired data and Wilcoxon's test.

Comparison of the final scores, active vs placebo achieved significance at 0.032 by T test or Wilcoxon's test 0.0457, see Table 3 and Figures 5 and 6. The erythema measurements and ultrasound recordings were not significantly different. Three of the twenty-two patients developed contact sensitivity to the topical preparations one to benzyl alcohol, one to sirolimus and one to both of these.

The antibody tests with Ki-67 showed a significant reduction of proliferating cells from a mean of 83/mm^3 in control to 55/mm^3 with Sirolimus (rapamycin) to give a significance of P-0.027 (T test). Using CD4 cells control values were 61/mm^3 against 32.7/mm^3 means values following rapamycin to give a significance of P-0.0026 (T-test). The T-test were unpaired due to missing samples.

Table 3 shows the clinical response to topical sirolimus. The clinical score is measured on a scale of 0-24 with higher values indicating a better result, ultrasound thickness in mm and erythema measurement in arbitrary units.

	Sirolimus		Control		Significance
	Mean	S.D.	Mean	S.D.	
Clinical Score	11.2	5.8	9.1	4.8	p=0.032
Ultrasound thickness	2.99	0.6	2.96	0.72	NS
Erythema measurement	34.5	7.9	33.1	7.7	NS

These results show that penetration of sirolimus from a formulation described above does occur. It is thought that increased adsorption would occur through the scalp to effectively treat scalp psoriasis.

CLAIMS:

1. A topical formulation for the treatment of a dermatological condition which comprises a macrocyclic lactone antibiotic, immunosuppressive macrolide or a pharmacologically active analogue, derivative or pro-drug thereof; characterized in that it further comprises a permeation modulator and the permeation modulator and the macrocyclic lactone antibiotic or macrolide or the pharmacologically active analogue, derivative or pro-drug thereof are present in relative amounts such that when a therapeutic amount is applied to the skin a minimal systemic effect is produced.

2. A formulation according to claim 1 comprising up to 10% by weight of the macrocyclic lactone antibiotic or the immunosuppressive macrolide or analogue, derivative or pro-drug thereof; the permeation modulator being present at 1 to 60% by weight.

3. A formulation according to either claim 1 or 2 wherein the macrocyclic lactone antibiotic is selected from erythromycin, azithromycin or clarithromycin.

4. A formulation according to either claim 1 or 2 wherein the immunosuppressive macrolide is selected from sirolimus, FK506 or SDZ ASM 981.

5. A formulation according to any preceding claim wherein the permeation modulator is an alkanoic acid or alkenic acid.

6. A formulation according to claim 5 wherein the alkanoic acid or alkenic acid is selected from capric acid, octanoic acid, oleic such acid or acids of intermediate chain length.

7. A formulation according to any preceding claim wherein the dermatological condition is selected from psoriasis, alopecia, eczema dermatitis, lichen planus, lupus erthematosus, pyoderma gangrenosum, vitiligo, graft versus host disease, pustular skin infections, bacterial skin infections or acne vulgaris.

8. A formulation according to claim 7 wherein the dermatological condition is eczema dermatitis and the concentration of macrocyclic lactone antibiotic or immunosuppressive macrolide is 0.05% to 2% by weight.

9. A formulation according to any preceding claim wherein the permeation modulator is used in conjunction with a solvent system.

10. A formulation according to claim 9 wherein the solvent system comprises an aromatic alcohol or a biologically acceptable benzene derivative, with or without an admixture of monoglycerides and/or a fatty acid ester.

11. A formulation according to either claim 9 or 10 wherein the permeation modulator comprises capric acid and the solvent system comprises benzyl alcohol.

12. A formulation according to any of claims 8 to 11 wherein the concentration of the solvent system is 5% to 90% by weight.

13. A formulation according to any preceding claim further comprising a thickening agent.

14. A formulation according to claim 13 wherein the

thickening agent is selected from white soft paraffin, cetostearyl alcohol, yellow soft paraffin, cetyl alcohol, steryl alcohol, divalent carboxylic acid soaps and carnauber wax.

15. A topical formulation for the treatment of a dermatological condition which comprises an immunosuppressive macrolide or a pharmacologically active analogue, derivative or pro-drug thereof; characterized in that it further comprises a permeation modulator; and the permeation modulator and the macrolide or the pharmacologically active analogue, derivative or pro-drug thereof are present in relative amounts such that when a therapeutic amount is applied to the skin a minimal systemic effect is produced.

16. A formulation according to either claim 15 wherein the immunosuppressive macrolide is selected from sirolimus, FK506 or SDZ ASM 981.

17. A formulation according to claim 16 wherein the immunosuppressive macrolide is sirolimus.

18. The use in the manufacture of a topical composition for the treatment of a dermatological condition of a macrocyclic lactone antibiotic or an immunosuppressive macrolide or a pharmacologically acceptable analogue, derivative or pro-drug thereof characterised in that it further comprises a permeation modulator and the permeation modulator; the macrocyclic lactone antibiotic or the immunosuppressive macrolide or pharmacologically acceptable analogue, derivative or pro-drug thereof being present in relative amounts such that when a therapeutic amount is applied to the skin a minimal systemic effect is produced.

19. The use of claim 18 wherein the macrocyclic lactone antibiotic or immunosuppressive macrolide is present at up to 10% by weight of the composition.

20. The use of an immunosuppressant macrolide, a macrocyclic lactone antibiotic or a pharmacologically active analogue, derivative or pro-drug thereof in the preparation of a topical formulation as claimed in any one of claims 1 to 17.

21. A method for the treatment of a disease of the skin or muccosa which comprises applying thereto a topical composition comprising a macrocyclic lactone antibiotic or an immunosuppressive macrolide or a pharmacologically acceptable analogue, derivative or pro-drug thereof; characterised in that it further comprises a permeation modulator; and the permeation modulator, the macrocyclic lactone antibiotic or the immunosuppressive macrolide or pharmacologically acceptable analogue, derivative or pro-drug thereof is present in relative amounts such that when a therapeutic amount is applied to the skin a minimal systemic effect is produced.

22. A method according to claim 21 wherein the macrocyclic lactone antibiotic or immunosuppressive macrolide is present at up to 10% by weight of the composition.

23. A method according to claim 21 or 22 wherein the immunosuppressive macrolide is utilized.

Figure 1

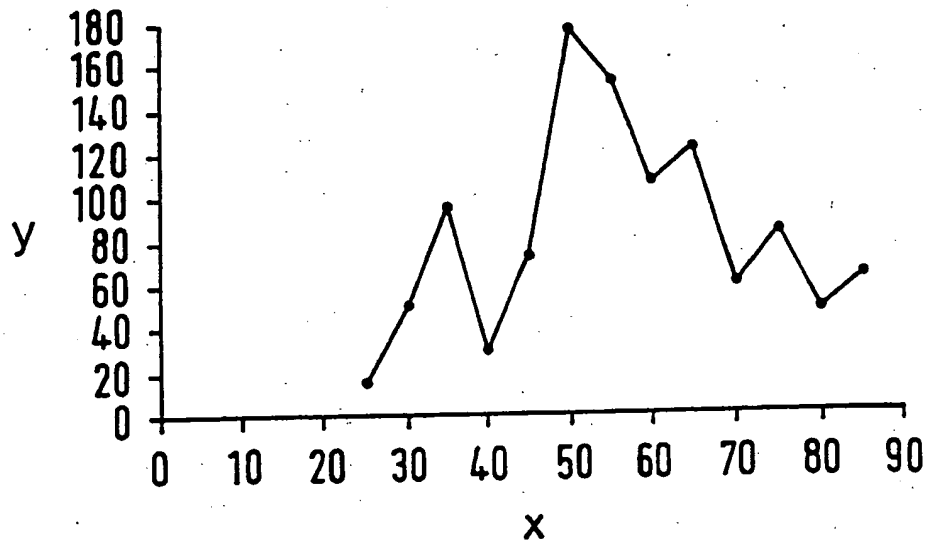


Figure 2

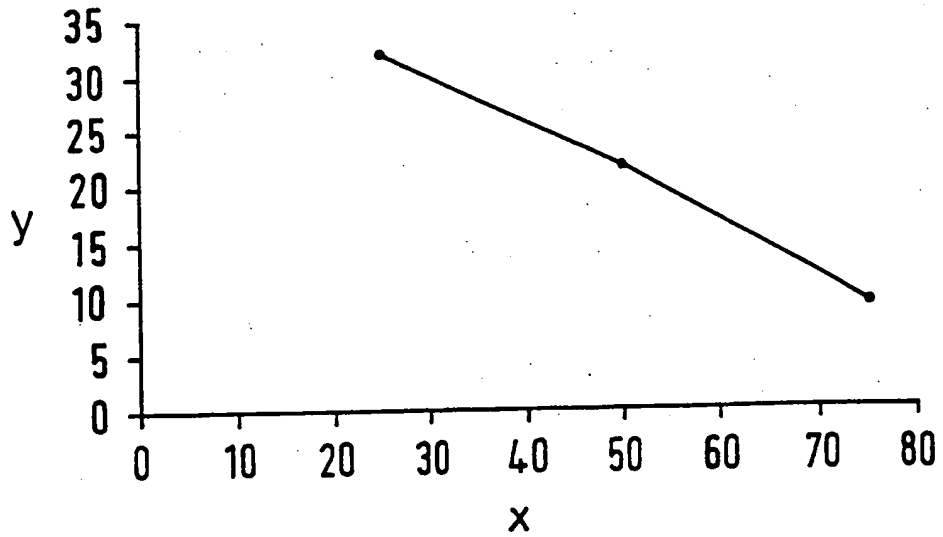


Figure 3

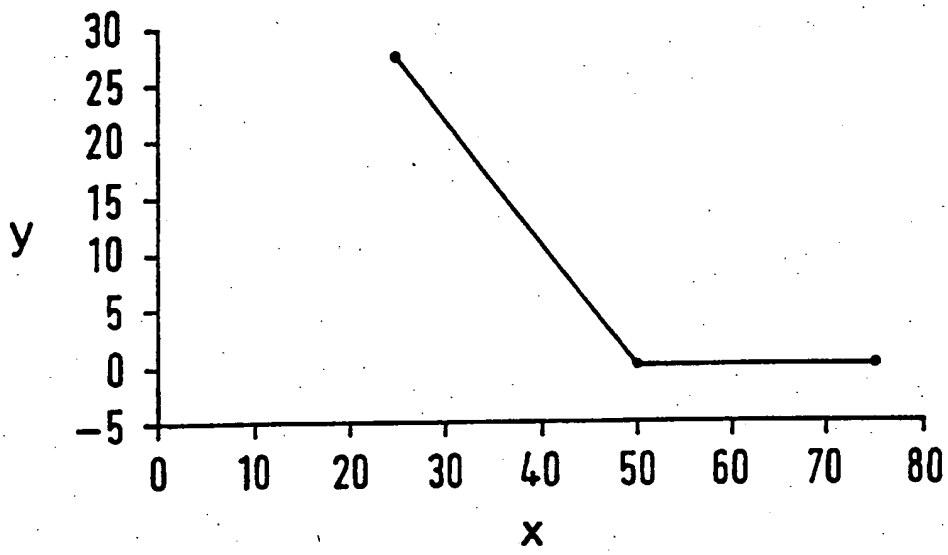


Figure 4

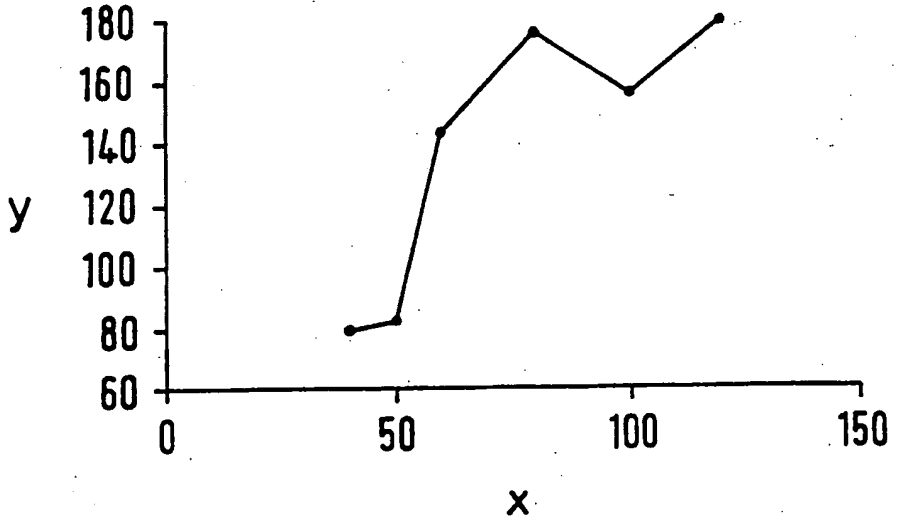


Figure 5

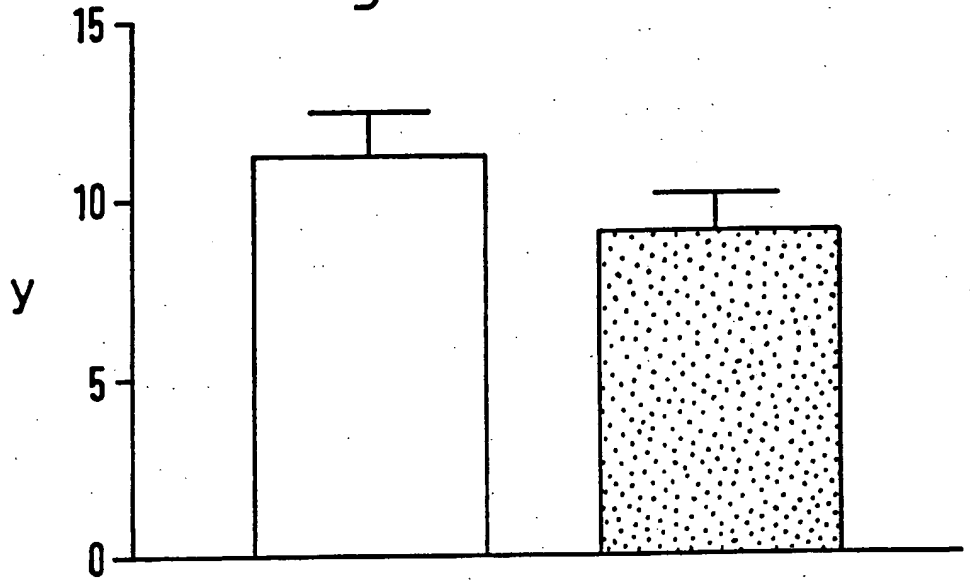
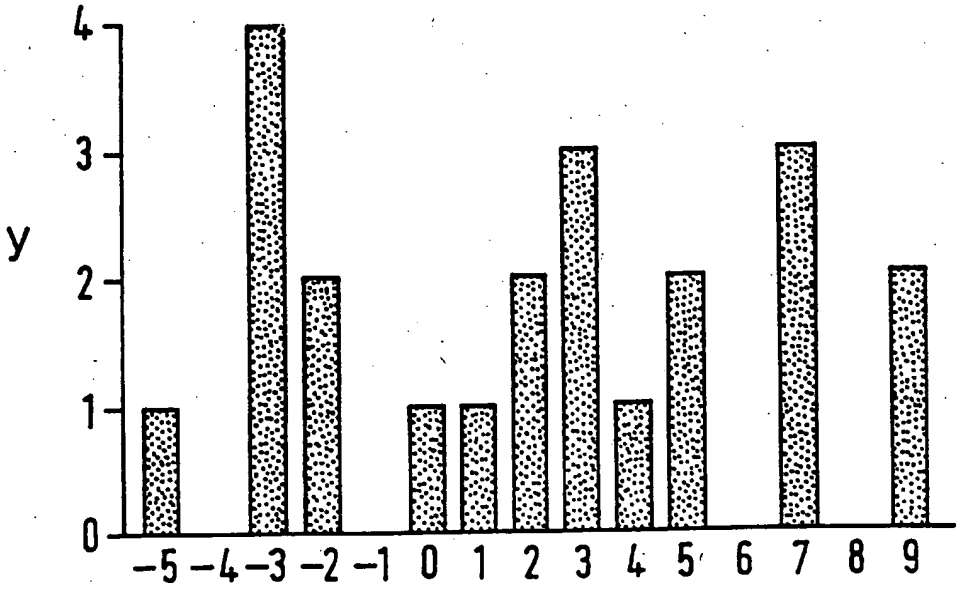


Figure 6



INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/GB 98/03317

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/445 A61K31/70 A61K38/13 A61K9/06 A61K47/12
A61K31/435

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Week 9631 Derwent Publications Ltd., London, GB; AN 96-306477 '31! XP002092952 see abstract & JP 08 133979 A (SANDO YAKUHIN KK,JP) 28 May 1996 ---	1,2,13, 15,18-23
A	EP 0 474 126 A (FUJISAWA) 11 March 1992 see claims see page 5, line 24 - line 42 ---	1-23
A	EP 0 582 239 A (RHONE-POULENC RORER) 9 February 1994 see claims see examples --- -/--	1-23

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 February 1999	18/02/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Scarponi, U

6

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/GB 98/03317

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	US 4 335 115 A (E.D.THOMPSON ET AL.) 15 June 1982 see claims	1-23
A	EP 0 027 286 A (PROCTER & GAMBLE) 22 April 1981 see claims see table 1 see examples	1-23
A	EP 0 753 297 A (FUJISAWA) 15 January 1997 see claims	1-23
A	WO 96 13249 A (SANDOZ) 9 May 1996 see claims	1-23
A	DE 44 18 115 A (SANDOZ) 1 December 1994 see claims	1-23
A	EP 0 273 202 A (E. VAN SCOTT ET AL.) 6 July 1988 see claims	1-23
A	EP 0 043 738 A (PROCTER & GAMBLE) 13 January 1982 see claims see page 6, line 23 - line 25	1-23
A	EP 0 435 436 A (PFIZER) 3 July 1991 see claims 1-5,7	1-23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 98/03317

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 21-23 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/GB 98/03317

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 474126 A	11-03-1992	AT 150304 T	15-04-1997
		AU 656145 B	27-01-1995
		AU 8351591 A	12-03-1992
		CA 2050623 A	05-03-1992
		CN 1059468 A	18-03-1992
		DE 69125230 D	24-04-1997
		DE 69125230 T	10-07-1997
		DK 474126 T	07-04-1997
		ES 2099112 T	16-05-1997
		GR 3022883 T	30-06-1997
		HK 1000006 A	03-10-1997
		JP 2526752 B	21-08-1996
		JP 5017481 A	26-01-1993
		PT 98862 A	31-08-1992
		SG 46547 A	20-02-1998
		RU 2079303 C	20-05-1997
		US 5385907 A	31-01-1995
EP 582239 A	09-02-1994	DE 4225697 A	10-02-1994
		DE 4323174 A	12-01-1995
		AU 4697393 A	03-03-1994
		CA 2120511 A	17-02-1994
		CN 1084742 A	06-04-1994
		WO 9403156 A	17-02-1994
		JP 7509001 T	05-10-1995
		MX 9304710 A	31-05-1994
		PL 302979 A	05-09-1994
US 4335115 A	15-06-1982	BE 860349 A	02-05-1978
		CA 1090253 A	25-11-1980
		DE 2748399 A	11-05-1978
		FR 2368949 A	26-05-1978
		GB 1587428 A	01-04-1981
		IE 45902 B	29-12-1982
		JP 53094030 A	17-08-1978
		NL 7712005 A,B,	03-05-1978
EP 0027286 A	22-04-1981	US 4299826 A	10-11-1981
		CA 1148469 A	21-06-1983
		JP 1018883 B	07-04-1989
		JP 1537607 C	16-01-1990
		JP 56099416 A	10-08-1981
EP 753297 A	15-01-1997	JP 6345646 A	20-12-1994
		AU 684286 B	11-12-1997
		AU 6816294 A	03-01-1995
		CN 1124925 A	19-06-1996
		WO 9428894 A	22-12-1994
WO 9613249 A	09-05-1996	AU 3845195 A	23-05-1996
		BR 9509530 A	14-10-1997
		CA 2200966 A	09-05-1996
		CZ 9701232 A	13-08-1997
		DE 19581804 T	22-01-1998
		EP 0786986 A	06-08-1997
		FI 971018 A	18-04-1997
		GB 2308546 A	02-07-1997
		HU 77140 A	02-03-1998

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/GB 98/03317

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9613249 A		JP 10508588 T	25-08-1998
		NO 971951 A	25-04-1997
		PL 319599 A	18-08-1997
		SK 52097 A	10-09-1997
		GB 2327610 A	03-02-1999
DE 4418115 A	01-12-1994	BE 1008329 A	02-04-1996
		CA 2124259 A	28-11-1994
		CH 686761 A	28-06-1996
		ES 2098180 A	16-04-1997
		FR 2705566 A	02-12-1994
		GB 2278780 A,B	14-12-1994
		IT 1272992 B	01-07-1997
		JP 7138161 A	30-05-1995
		GB 2315216 A,B	28-01-1998
EP 273202 A	06-07-1988	AU 654850 B	24-11-1994
		AU 1394392 A	28-05-1992
		AU 618517 B	02-01-1992
		AU 7998687 A	23-06-1988
		CA 1324077 A	09-11-1993
		CA 1339706 A	10-03-1998
		DE 3751361 D	27-07-1995
		DE 3752045 D	07-05-1997
		DE 3752045 T	13-11-1997
		EP 0599819 A	01-06-1994
		EP 0770399 A	02-05-1997
		ES 2074978 T	01-10-1995
		ES 2103506 T	16-09-1997
		JP 2533339 B	11-09-1996
		JP 63166837 A	11-07-1988
		US 5665776 A	09-09-1997
		US 5389677 A	14-02-1995
		US 5702688 A	30-12-1997
		US 5422370 A	06-06-1995
		US 5470880 A	28-11-1995
		US 5547988 A	20-08-1996
		US 5091171 A	25-02-1992
		US 5561159 A	01-10-1996
		US 5591774 A	07-01-1997
		US 5550154 A	27-08-1996
		US 5589505 A	31-12-1996
		US 5561155 A	01-10-1996
		US 5670541 A	23-09-1997
		US 5668177 A	16-09-1997
		US 5827882 A	27-10-1998
		US 5580902 A	03-12-1996
		US 5670542 A	23-09-1997
		US 5612376 A	18-03-1997
		US 5674899 A	07-10-1997
		US 5643961 A	01-07-1997
		US 5648395 A	15-07-1997
		US 5643962 A	01-07-1997
		US 5643952 A	01-07-1997
		US 5656665 A	12-08-1997
		US 5677339 A	14-10-1997
		US 5574067 A	12-11-1996
		US 5650436 A	22-07-1997

INTERNATIONAL SEARCH REPORT

..formation on patent family members

Inter ›nal Application No
PCT/GB 98/03317

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 273202 A		US 5637615 A	10-06-1997
		US 5643953 A	01-07-1997
		US 5556882 A	17-09-1996
		US 5554651 A	10-09-1996
		US 5583156 A	10-12-1996
		US 5654340 A	05-08-1997
		US 5677340 A	14-10-1997
		US 5674903 A	07-10-1997
EP 0043738 A	13-01-1982	AU 544969 B	27-06-1985
		AU 7272081 A	14-01-1982
		CA 1165240 A	10-04-1984
		IE 51377 B	10-12-1986
		JP 1737953 C	26-02-1993
		JP 4020886 B	07-04-1992
		JP 57081408 A	21-05-1982
		US 4954487 A	04-09-1990
		ZA 8104650 A	28-07-1982
EP 435436 A	03-07-1991	US 5023085 A	11-06-1991
		AU 613281 A	25-07-1991
		CA 2030943 A	30-05-1991
		DE 69006000 D	24-02-1994
		DE 69006000 T	05-05-1994
		DK 435436 T	28-02-1994
		IE 63597 B	17-05-1995
		IL 96449 A	23-07-1996
		JP 1955584 C	28-07-1995
		JP 3176426 A	31-07-1991
		JP 6088912 B	09-11-1994
		PH 27105 A	16-03-1993
		PT 96021 A,B	13-09-1991

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☑ FADED TEXT OR DRAWING
- ☑ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.